ABSTRACTS

ISCFR 2012

July 26-29, Whistler, Canada

7th International Symposium on Canine and Feline Reproduction

In a joint meeting with

EVSSAR 2012

15th Congress of the European Veterinary Society for Small Animal Reproduction

Editors: Gary England, Michelle Kutzler, Pierre Comizzoli, Wojciech Nizanski, Tom Rijsselaere and Patrick Concannon

Reprinted in IVIS with the permission of the ISCFR Organizers
Effect of natural photoperiod on epididymal sperm quality and testosterone serum concentration in domestic cat (*Felis catus*)

Nuñez Favre, R1,2; Bonaura, MC1,3; Tittarelli, CM1; Mansilla Hermann, D1; de la Sota, RL1,2 and Stornelli, MA1

1Catedra y Servicio de Reproducción Animal, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, La Plata B1900AVW, Buenos Aires, Argentina; 2CONICET, Av. Rivadavia 1917, Capital Federal C1033AAJ, Argentina and 3CIC Calle 526 e/10 y 11, La Plata B1906AOM, Buenos Aires, Argentina

astornel@fcv.unlp.edu.ar

INTRODUCTION: The queen is a seasonal breeder when exposed to natural photoperiod, with ovarian activity ceasing under decreasing photoperiod and resuming with increasing photoperiod. This seasonality may be observed in both females and males of several mammalian species (1,2), and in these species, photoperiod and melatonin concentrations are related to circannual sperm production. Previous studies have shown seasonal changes in testicular cell morphology in domestic cats (3). Probably these changes are reflected in seasonal variations of epididymal sperm quality. The aim of this study was to assess epididymal sperm characteristics and serum testosterone concentration in cats under natural photoperiod. Our hypothesis was that natural photoperiod induces seasonal changes in spermatozoa quality and serum testosterone concentration.

MATERIALS AND METHODS: Forty eight privately-owned short-hair mixed breed male cats, aged between 1 to 5 years were included in this study. Cats included were those included in a program for breeding control at a municipal pet public shelter. Before surgery, all animals were anesthetized with a combination of ketamine (25 mg/kg i.m.; Vetanarcol®, Laboratorios Koning SA, Argentina), xylazine (1 mg/kg i.m.; Sedomin®, Laboratorios Koning SA, Argentina). After surgery, all animals were medicated for 3 days with Amoxicilin (22 mg/kg i.r.n., Argentina) and Tramadol (1mg/kg i.m., Algen® Laboratorio Richmond, Argentina). All surgical procedures were performed by a licensed veterinarian and followed approved guidelines for ethical treatment of animals (ref). To show the true effect of season, only toms castrated during the two last weeks of each season were included in the study. Hence, according the time of castration, epididymis were divided into two groups, group I toms castrated during increasing light (IL; [winter and spring; IL, n=24]), group II toms castrated during decreasing light (DL; [summer and fall; IL, n=24]). After orchiectomy, each testis with adjacent epididymis was transported to the laboratory in saline solution. Right and left epididymides were processed separately and epididymal sperm recovery was made within 1-2 h after surgery. For recovering epididymal sperm, the cauda epididymis was first carefully separated from the testis and deferens conduct and placed in a glass tube containing 0.75 ml TRIS solution (3.025 g TRIS, 1.70 g citric acid, 1.25 g fructose, distilled water added up to 100 mL). After 10 minutes at 37ºC, sperm samples were obtained by cutting the cauda epididymis in TRIS solution (3.025 g TRIS, 1.70 g citric acid, 1.25 g fructose, distilled water added up to 100 mL). After 10 minutes at 37ºC, sperm samples were obtained by cutting the cauda epididymis in TRIS solution. The following tests were performed on sperm samples: motility (MOT, % motile), velocity (VEL, 0-5), total sperm cells (TS, 106), acrosome integrity (ACR, % spermatozoa with intact acrosome). Labortorie Richmond, Argentina). All surgical procedures were performed by a licensed veterinarian and followed approved guidelines for ethical treatment of animals (ref). To show the true effect of season, only toms castrated during the two last weeks of each season were included in the study. Hence, according the time of castration, epididymis were divided into two groups, group I toms castrated during increasing light (IL; [winter and spring; IL, n=24]), group II toms castrated during decreasing light (DL; [summer and fall; IL, n=24]). After orchiectomy, each testis with adjacent epididymis was transported to the laboratory in saline solution. Right and left epididymides were processed separately and epididymal sperm recovery was made within 1-2 h after surgery. For recovering epididymal sperm, the cauda epididymis was first carefully separated from the testis and deferens conduct and placed in a glass tube containing 0.75 ml TRIS solution (3.025 g TRIS, 1.70 g citric acid, 1.25 g fructose, distilled water added up to 100 mL). After 10 minutes at 37ºC, sperm samples were obtained by cutting the cauda epididymis in TRIS solution. The following tests were performed on sperm samples: motility (MOT, % motile), velocity (VEL, 0-5), total sperm cells (TS, 106), acrosome integrity (ACR, % intact; FITC-PSA), plasma membrane integrity (MI, %intact; CFDA-PI) and sperm morphology (SM, % normal; Tincion 15°, Biopur). Before bilateral orchiectomy blood samples were taken to measure serum concentrations of testosterone (T2). All samples were centrifuged and stored at –20 ºC until T 2 were measured by a solid-phase radioimmunoassay (RIA) using 1I25 (Coat-A-Count, total testosterone; Diagnostic Product Corporation, Los Angeles, CA, USA). Data are presented with the Mixed procedure of SAS. Hence, sperm quality and testosterone concentrations of toms castrated in days with increasing light (IL, 9h 51’ to 14h 27’ daylight; Group I) were compared with toms castrated in days with decreasing light (DL, 14h 27’ to 9h 51’ daylight; Group II). Data are presented as LSM ± SEM. Significance was defined as P < 0.05.

RESULTS: Toms castrated during IL had higher plasma membrane integrity and sperm morphology compared to toms castrated during DL (69.0 ± 2.7 vs. 60.6 ± 2.1, P<0.01; 45.9 ± 2.5 vs. 35.9 ± 3.4; P<0.02; respectively). Similarly, toms castrated during IL tended to had higher motility and total sperm cells compared to toms castrated during DL (56.3 ± 2.8 vs. 47.3± 3.7, P<0.06; 13.8 ± 1.4 vs. 10.0 ± 1.8, P<0.09). However, velocity and acrosome integrity were similar between both groups (3.5 ± 0.1 vs. 3.4 ± 0.1, P>0.6; 45.8 ± 3.3 vs. 44.0 ± 4.0, P>0.72; respectively). Furthermore, serum testosterone concentrations were similar between toms castrated in IL vs. toms castrated in DL (0.76 ± 0.15 vs. 0.59 ± 0.19, P>0.51).

DISCUSSION: Recent studies have shown seasonal changes in testicular cell morphology in domestic cats (3). Stornelli et al. (3) found that testicles from tom cats orqueietomized during long hours days had higher percentage of tubules with tailed and mature sperm ready for release from Sertoli cells compared to testicles from males orqueietomized during short hours days. Similarly, it has been reported that the number of sperm collected by electro-ejaculation was different between the non-breeding seasons and breeding seasons of male cats in Australia (4). In agreement with this study, Axner and Linde Fosberg (5) found a higher percentage of normal spermatozoa during February to July than during August to January. Similarly, Blottner and Jewgenow (6) found that testis weight and the total sperm number per testis showed significant
differences between spring and autumn. These findings support our result in which we found a tendency to have higher motility and total sperm cells in increasing light compared with decreasing light. The range of testosterone blood concentration found in toms castrated in IL in this work was similar to those reported by Tsutsui et al. (7) during the breeding season (0.25-2.5 ng/mL, with an average of 1.69 ng/mL). Whereas, Johnstone et al. (4) reported that testosterone levels were greatly affected by the season; Kirkpatrick et al. (8) reported hardly any differences between breeding and non-breeding seasons in agreement with the results from this study.

CONCLUSION: We conclude that natural photoperiod induces seasonal changes in spermatozoa quality but not in serum testosterone concentrations.